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Maturation performance of *Penaeus vannamei* co-fed *Artemia* biomass preparations

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Abstract

Few shrimp hatcheries successfully propagate captive broodstock on a commercial scale. Diets for acceptable maturation performance of *Penaeus vannamei* have typically relied on the inclusion of marine polychaetes (bloodworm) from Panama or Maine, USA, which are expensive and are of unpredictable supply. Studies were therefore undertaken at experimental and commercial scale to replace or supplement the polychaetes by frozen ongrown *Artemia* which were either non-enriched or bioencapsulated with specific boosters. In experiment 1 the control diet consisted of frozen squid only and was evaluated against broodstock diets where 60% of the squid was substituted by bloodworm or enriched *Artemia* biomass. Effects were evaluated for males and females as sexes were kept in separate broodstock tanks. In experiment 2 the control treatment received a mixture of natural feed, including bloodworm. For the two other treatments, the bloodworm fraction was replaced by non-enriched and enriched *Artemia* adults, respectively. Finally, an evaluation at commercial scale was made comparing two feeding regimes based on a combination of semi-moist pellets and fresh-frozen marine organisms. In one of the treatments a portion of the natural food was replaced by an equal amount of enriched, frozen *Artemia* biomass. Dietary effects on the reproductive performance of the broodstock were evaluated and egg characteristics were monitored. Results from the three experiments confirm that dietary conditions affect the reproductive performance of *P. vannamei*. Even in a maturation diet consisting of

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multiple natural food products, frozen adult brine shrimp biomass appears to increase reproductive performance. Although the male diet did not appear to significantly affect mating or fertilization, there was a clear tendency towards improved mating success and hatching when *Artemia* biomass was included in a mixed diet. Combined effects of improved mating and hatching resulted in significant differences in overall nauplii production. It is therefore concluded that *Artemia* biomass may be useful as a supplement to or as a replacement for polychaetes in *Penaeus vannamei* maturation diets. © 1997 Published by Elsevier Science B.V.

Keywords: Penaeid maturation; Broodstock; *Artemia* biomass; Nutrition; *Penaeus vannamei*

1. Introduction

In penaeid shrimp, nutritional factors play a critical role in the stimulation of sexual maturation and mating, the enhancement of fertility, and the viability and quality of offspring (Harrison, 1990). Broodstock animals are typically fed a combination of natural and dry feeds or semi-moist pellets. Feeding regimes made up of combinations of various components have always outperformed those where one of the ingredients was offered singly (Chamberlain and Lawrence, 1981; Galgani et al., 1989; Bray et al., 1990). Many maturation system managers consider marine polychaetes (bloodworms) and mainly originating from Maine, USA (*Glycera dibranchiata*) or Panama (*Americonuphis reseii*) essential for successful nauplii production in *Peneaus vannamei* (Browdy, 1992). Bloodworm supplementation has been reported to increase reproductive performance (Gomez and Arellano, 1987) and it has been suggested that certain polyunsaturated fatty acids in bloodworms may help trigger maturation (Middleditch et al., 1979, 1980; Lytle et al., 1990).

However, bloodworms are often the most expensive component of the maturation diet comprising a significant portion of overall maturation system cost (Rhodes et al., 1992). Therefore, reducing dependence on bloodworms provides economic benefit to the shrimp industry. Moreover, their supply is unpredictable and, along with other natural feeds, their biochemical composition may vary according to location and season of collection as well as the method and duration of storage (Lytle and Lytle, 1990).

Experience with *Artemia* biomass as a dietary component in shrimp maturation is scarcely documented. Bray et al. (1990) demonstrated that administration of fresh-frozen squid, bloodworm (*Americonuphis* sp.), shrimp and whole adult *Artemia* in a ratio 4:2:2:1 to *P. stylirostris* resulted in a good reproductive performance. Leung-Trujillo and Lawrence (1991) fed a combination of 12% adult *Artemia* together with 22% each of squid, bloodworm, shrimp head and dry feed. In both cases, no specific *Artemia*-related effects were reported. On the other hand, Browdy et al. (1989) found an enhanced reproductive performance when using frozen *Artemia* as a dietary supplement for *P. semisulcatus*. The reproductive activity was however not consistent and this was attributed to possible variability in the nutritional quality of the different batches of brine shrimp used. When using live adult *Artemia* it is possible to boost the *Artemia* with nutritional supplements (Lavens and Sorgeloos, 1996), which may stimulate reproductive performance in shrimp. These may include $n-3$ highly unsaturated fatty acids (HUFA), vitamins or astaxanthin. Such a bioencapsulation technique should reduce variability among *Artemia* batches and enhance nutritional quality.

The objective of the present study was to evaluate the potential of *Artemia* (whether or not enriched with maturation enhancing components) as a possible replacement for marine polycphaetes in the maturation diet of *P. vannamei*.

2. Material and methods

2.1. Experiment 1

A first trial was run in the experimental facilities of the Waddell Mariculture Center (WMC), SC, USA. Six round maturation tanks (4.3 m diameter, 76 cm water depth) were operated according to the unisex system as described in Browdy et al. (1996). Sixty males or females were stocked in each tank with a total of three tanks per sex. Highly turbid water pumped from an estuarine source was sand filtered and settled for 48 h while passing through a series of twelve 41.5-m^3 tanks. After final filtration with a modified quartz media filter system (Diamond Water Systems, Holyoke, MA, USA) and a diatomaceous earth filter (Nautilus FNS-60, Pac Fab, Sanford, NC, USA), the water was heated to 29°C and distributed to the tanks at an exchange rate of 200% $\times$ day^{-1}. The temperature in the maturation tanks averaged 28.1 $\pm$ 1.1°C. The tanks were submitted to a 13:11 h light:dark photoperiod.

Specific pathogen free (SPF) *P. vannamei* broodstock (Wyban et al., 1993) were obtained from the Oceanic Institute, and maintained in a holding facility at WMC for 6 months before transfer to the maturation tanks. Four days per week, 1 or 2 h before dusk, females were checked for gonadal development and fully developed females were sequentially transferred to one of the three all-male tanks for mating. One to two hours after dusk, these females were examined for evidence of mating. Mated females were removed and placed individually in 200-l spawning tanks. The next morning, females were returned from the spawning tanks to the respective maturation tank following examination of the ovaries for completeness of spawning. Two to three 200-ml aliquot samples of the spawned eggs and the hatched nauplii were taken from each spawning tank and examined under a stereoscope to determine spawn size, percent fertility and percent hatch. Those females which did not mate were left in the male tank until either spawning or re-absorption was noted, and then returned to their maturation tank. Effluents from all maturation tanks were sampled daily for fertile or non-fertile spawns. Uneaten food or faecal material was siphoned from the maturation tanks daily.

Three frozen diets were tested, such that each diet was fed to one male and one female tank. The control diet consisted of squid at a feeding rate of 25% of the tank biomass (wet weight, WW) per day, the second diet of squid and polychaetes (*G. dibranchiata*) at 10% and 15% respectively of tank biomass per day, and the third diet of squid and enriched *Artemia* biomass (commercial product obtained from San Francisco Bay Brand, Newark, CA, USA), both at 15% of tank biomass per day. The initial amount of *Artemia* in the diet was higher than the bloodworms (15% instead of 10%) as preliminary tests have indicated that it had a somewhat greater tendency to wash out of the tank. Squid was administered to all tanks during the evening feeding whereas during the morning feeding (8:30 a.m.) squid, bloodworm or *Artemia* was

respectively offered. At the same time water exchange was discontinued for 1 h in order to avoid food losses with the effluent water. Feeding rates were adjusted over the course of the experiment such that diets were consumed ad libitum.

2.2. Experiment 2

The second trial on an experimental scale was executed at the Centro Nacional de Acuicultura e Investigaciones Marinas (CENAIM) in Ecuador. Mixed populations of wild caught *P. vannamei* were kept in oval-shaped tanks (5 m × 3 m, water depth 70 cm) at a density of 4 animals × m^{-2}, or a total of 63 shrimp per tank with a male-to-female ratio of 1.1:1. The experimental animals originated from two locations 400 km apart along the Ecuadorian coast (Esmeraldas province, North Ecuador and Guayas province, South Ecuador). The Esmeraldas animals arrived at the Center in five different shipments, the Guayas ones arrived as one batch. Upon arrival all animals were acclimated in indoor tanks for three to five weeks depending on their time of arrival at the Center. During this period heavy mortality occurred (about 50%) but no causal factors could be detected. One week following the stabilization of the survival the females were unilaterally ablated. Together with the males they were then randomly transferred to one of the three experimental tanks, maintaining similar ratios of the two origins in each tank. By exchanging the sand-filtered and preheated ocean water (34 ppt salinity) at a rate of 200% × day^{-1} the water temperature in the tanks was maintained at 28 ± 0.5°C. The broodstock was submitted to an inversed photoperiod of 14 h light with gradual transition between light and dark hours.

During the first two hours after dusk the tanks were inspected and mated females were transferred to spawning tanks of 500 l capacity filled with sand-filtered ocean water kept at 28°C. Upon spawning, the animals were returned to their respective maturation tanks and the eggs were harvested, counted and then re-suspended in 500 l of sea water (28°C) for hatching. Effluents from all tanks were sampled daily for possible occurrence of fertile or non-fertile spawns in the maturation tanks. Uneaten food or faecal material was siphoned from the maturation tanks daily.

Different feeding regimes, based on a mixture of fresh-frozen marine organisms (squid, oyster, mussel), were administered in five daily rations to the maturation tanks. Feeding regime 1 (control) consisted of this mixture together with bloodworm (*A. reseii*) at 12% and 5% respectively of tank biomass per day. In feeding regimes 2 (Art) and 3 (ArtE) the bloodworm fraction was substituted on a dry weight basis by non-enriched or enriched *Artemia* biomass, respectively. At the time of *Artemia* feeding the water exchange was discontinued for 1 h in all tanks to prevent *Artemia* losses. The *Artemia* biomass was obtained from outdoor culture ponds (120 ppt salinity) and transported to CENAIM for enrichment and/or freezing. The enrichment procedure consisted of acclimation to normal sea water (35 ppt) for 5 h, overnight storage in holding tanks of 4000 l capacity at densities of about 1000 *Artemia* × l^{-1} while feeding on *Tetraselmis* sp. Enrichment was in 500 l tanks at densities of 5000 *Artemia* × l^{-1} using an experimental self-emulsifying concentrate containing high levels of DHA-oil, cholesterol, vitamin C and E, and astaxanthin. The tanks received two doses of enrichment product (100 ppm per dose): one at the start and a second one 2 h later. After

4 h of enrichment the brine shrimp were harvested, rinsed in sea water and fresh water, and frozen in 1-cm thick blocks of 300 g (moisture content 90%) at −22°C.

Penaeid shrimp tissue was analysed at the beginning (three females) and at the end (three mature, unmated females for each treatment) of the experiment. Gonads and midgut glands were removed, pooled per treatment and preserved/stored at −85°C for lipid analysis. Batches of nauplius-V stages originating from parents that had matured in captivity as well as in the natural environment were also sampled. The analyses were carried out on duplicate samples. Lipid extraction followed the method described by Folch et al. (1957). Fatty acids (FA) were saponified with a methanolic sodium hydroxide solution (NaOH 0.5 N in methanol) and esterified by adding 2 ml of a boron trifluoride methanol complex (14%BF_3MeOH). The fatty acid methyl esters (FAME) were analyzed using a gas chromatograph (Shimadzu GC-14A) provided with a flame ionization detector. FAME were separated on a glass column (2.1 m × 3.2 mm) packed with GP10% SP2330. The column was set to a programme temperature of 160 to 225°C (3°C × min^{-1}); the temperatures of injector and detector were kept at 250°C. Nitrogen was used as gas carrier. FA were identified by comparison between different retention times of PUFA-1 and PUFA-2 qualitative standards. For the quantitative analysis use was made of GLC-10, RM-3 and rapeseed oil (Supelco) standards which contain saturated and unsaturated fatty acid mixtures.

2.3. Experiment 3

This experiment was performed within the facilities of Aqualab S.A., a commercial hatchery in the Guayas province, Ecuador. The trial was executed at production scale according to the routine procedures of Aqualab; this includes the application of an inversed photoperiod and artificial insemination of mature females. 24 tanks (4 m diameter; stocking density of 80 female shrimp) were divided into two groups. One group received the control diet consisting of 3.6% of a home-made maturation pellet and 14.4% of a mixture of fresh-frozen marine organisms (squid, mussel, oyster) but not containing bloodworm. The other group was fed a modified diet in which 14% of the natural ingredients were replaced by pond-reared *Artemia* biomass enriched with Aqualab's home-made formula (production, harvesting, enrichment, freezing and storage procedures for the *Artemia* biomass were identical to the ones applied in experiment 2). The feeding regime consisted of five feeding rations × day^{-1}.

Reproduction parameters were checked in the same way as described for the other experiments.

2.4. Statistical evaluation

Operation of experimental systems for controlled maturation and reproduction of penaeid shrimp is resource intensive. The availability of labor, facilities and supplies limit the potential for replication. These limitations must be considered carefully in interpreting results. In the present study, the assumption was made that shrimp in a given tank were representative of a population subjected to a specific treatment. Due to system limitations in the two experimental set-ups, each diet was tested in one tank

without replication. To control for tank effects in the WMC-experiment each population was moved every 14 days to a cleaned tank in a sequential order. At the end of the 12-week experiment each group had been in each tank for a two week period. Gender-specific logistic regression models were used to examine mating and fertilization success as influenced by the independent variable of diet. Other data were analyzed using the GLM procedure for analyses of variance (SAS Institute, 1988). In the CENAIM-experiment the data were analyzed using one-way ANOVA and Scheffe's multiple range test ($p < 0.05$). For the statistical analyses of the percent data obtained in experiments 1 and 2 arcsin transformations were applied.

3. Results

3.1. Experiment 1

Average initial weight of stocked male shrimp was 45 g: 44.9 ± 3.8 g (control diet), 44.6 ± 3.4 g (bloodworm diet), and 45.5 ± 4.5 g (*Artemia* diet). Average female weight at stocking was about 25% higher (55 g): respectively 54.1 ± 8.9 g, 55.8 ± 5.2 g, and 55.4 ± 5.2 g. Survival rates for females dropped continuously and were finally relatively low in all treatments, i.e. 7% (squid diet), 22% (bloodworm diet) and 36% (brine shrimp diet). The female tank fed bloodworm was accidentally drained for a short time on day 62 resulting in the loss of 10 animals in 3 days. Male survival was high during the first half of the experiment dropping to 34% (squid diet), 28% (bloodworm diet), and 30% (*Artemia* diet) at the end of the 12-week experiment. Mortality was often associated with tank transfers. Females fed various diets were transferred sequentially into male tanks which in turn received one of the three different diets. Before exploring differences between diets in male and female reproductive performance, tests were carried out to evaluate the level of interaction between male and female diet, and between diet and possible tank effects. No significant interactions were found ($p > 0.05$).

Mature females (stage IV) were transferred to male tanks for mating a total of 95 times for the control treatment, 137 times for the bloodworm treatment, and 158 times for the brine shrimp treatment, reflecting a trend towards increased ovarian development in the *Artemia* treatment (Table 1a). In addition to the increased development, females fed bloodworm or *Artemia* were more likely to mate and produce fertile spawns than females fed squid only. Females fed *Artemia* had significantly higher mating rates than those fed only squid ($p < 0.05$).

In evaluating spawns from females fed each of the three diets, no significant differences were found in spawn size ($p > 0.05$, Table 1a). However, the percent fertilization for the fertile spawns, number of fertile eggs per spawn, and the number of nauplii produced per female per day were significantly greater when a squid diet was supplemented with *Artemia* ($p < 0.05$). Although a trend towards increased reproductive performance was observed for the latter, the only significant difference between bloodworm and *Artemia* supplements was for the average number of nauplii produced per female per day ($p < 0.05$, Table 1a).

Table 1
Experiment 1: Reproductive performance of *P. vannamei* females (a) and males (b) fed squid versus squid supplemented with Maine bloodworms or *Artemia* biomass enriched with a commercial emulsion [1]

	Dietary supplement		
	Squid	Squid + bloodworm	Squid + *Artemia*
(a) Females			
Number of transfers	95	137	158
Mating success (%)	13.7 [b]	23.4 [a,b]	25.5 [a]
Fertilization success (%)	8.4 [a]	11.7 [a]	15.2 [a]
Total eggs/spawn (×1000)	117.0 [a]	115.6 [a]	130.6 [a]
Percent fertilization [2]	25.9 [b]	34.4 [ab]	51.2 [a]
Fertile eggs/spawn (×1000)	29.4 [b]	42.8 [a,b]	70.3 [a]
Nauplii/female/day	65 [b]	195 [b]	453 [a]
(b) Males			
Number of transfers	130	132	128
Mating success (%)	26.4 [a]	16.7 [a]	22.7 [a]
Fertilization success (%)	14.6 [a]	8.3 [a]	14.1 [a]
Percent fertilization [2]	31.9 [b]	38.7 [a,b]	41.0 [a]
Fertile eggs/spawn (×1000)	30.6 [b]	56.6 [a,b]	55.3 [a]

[1] Numbers with similar superscripts within rows are not significantly different ($p > 0.05$).
[2] The results were arcsin transformed for statistical tests but are here presented as untransformed means.

The number of developed females transferred into each male tank was similar in all treatments (130, 132, and 128 for control, bloodworm and *Artemia* diet, respectively). No significant differences were found in mating success, fertilization success, percent fertilization of individual spawns, or in the number of fertile eggs per spawn ($p > 0.05$, Table 1b).

3.2. Experiment 2

Overall survival was high in all treatments with slightly inferior results for the control treatment (Table 2). The experimental design, however, did not allow statistical analyses for significance of observed variations. Males showed higher survival rates than females resulting in a slowly increasing male-to-female ratio over the course of the experiment.

Table 2
Experiment 2: Survival of *P. vannamei* fed a mixture of marine organisms supplemented with Panama bloodworm (control), non enriched *Artemia* biomass (Art) or *Artemia* biomass enriched with an experimental emulsion (ArtE)

Treatment	Males			Females		
	Animals		Survival (%)	Animals		Survival (%)
	Stocked	Harvested		Stocked	Harvested	
control	28	25	89.3	26	19	73.1
Art	32	31	96.9	30	25	83.3
ArtE	32	32	100	29	26	89.7

Table 3
Experiment 2: Maturation efficiency and mating success of *P. vannamei* fed a mixture of marine organisms supplemented with Panama bloodworm (control), non enriched *Artemia* biomass (Art) or *Artemia* biomass enriched with an experimental emulsion (ArtE)

Maturation stage	Dietary supplement		
	Control	Art	ArtE
Gonadal development (cumulated)	346	401	348
Maturation efficiency [1, 2]	25.7 [a]	24.9 [a]	21.3 [a]
Spawns (cumulated)	20	62	40
Daily mating success [1, 2]	6.1 [a]	16.2 [b]	11.3 [a,b]
Population efficiency			
Surviving females spawned (%)	73.7	88.5	84.0
number of spawns per surviving female	1.05	2.38	1.68
spawning frequency per surviving female	2.9	0.94	1.45
Eggs per spawn (× 1000) [1]	227.8 [a]	247.1 [a]	243.1 [a]
Hatching percentage [1, 2]	43.1 [a]	40.5 [a]	46.3 [a]
Nauplii per spawn (× 1000) [1]	107.4 [a]	105.7 [a]	120.5 [a]
Nauplii per night per female [1]	1582 [a]	4127 [b]	2805 [a,b]

[1] Numbers with similar superscripts within rows are not significantly different ($p > 0.05$).
[2] The results were arcsin transformed for statistical tests but are here presented as untransformed means.

This change in sex ratio occurred in a similar manner in all treatments and it can reasonably be assumed that any possible effect on the mating efficiency of mature females was comparable in all tanks.

Females with developed gonads were recorded a total of 346, 401 and 348 times for the control, Art and ArtE, respectively (Table 3). These values correspond with maturation efficiencies of 25.7%, 24.9% and 21.3% respectively, and did not reveal significant treatment effects ($p > 0.05$, Table 3). Of the ripe females (stage IV) respectively 20, 62 and 40 of them actually mated and were subsequently transferred to the spawning tanks. The daily mating success of mature females in the control tank was 6.1%; this was significantly inferior to the 16.2% for the tank fed non-enriched brine shrimp ($p < 0.05$, Table 3). The intermediate result (11.3%) of the tank fed enriched *Artemia* could not be differentiated statistically from either extreme ($p > 0.05$, Table 3). Table 3 further illustrates that the fraction of the surviving female population that produced at least one spawn is larger in the *Artemia* supplemented feeding regimes (88.8% in Art and 84.0% in ArtE) than in the control having a bloodworm supplementation (73.7%). *Artemia* supplementation also scored better than bloodworm supplementation as far as the number of spawns per female and the spawning frequency are concerned (Table 3).

No treatment effects could be detected for spawn size (number of eggs per spawn) which reached high values of 227,800 in the control treatment, 243,100 in the non-enriched and 247,100 in the enriched *Artemia* treatments ($p > 0.05$, Table 3).

In this experiment the hatching percentage was calculated as an indication of egg viability. For this variable no significant variation could be detected among treatments ($p > 0.05$, Table 3). As a consequence the average number of nauplii hatching out of

individual spawns is statistically not different among treatments. The average number of nauplii produced per night and per female are 1582 for control, 4127 for Art, and 2805 for ArtE. This variable combines both spawn size and mating success of which only the latter showed a pronounced and significant treatment effect. The average number of nauplii per night and per female therefore shows significant variation similar to the one observed for the mating success ($p < 0.05$, Table 3).

Data on the lipid concentration and the fatty acid composition of the different dietary components and specific shrimp tissues are summarized in Table 4. Little variation is observed in the lipid levels of the different natural food components: only the enriched *Artemia* contained a higher lipid content, i.e. 17% instead of 12%. Lipid levels in the ovaries of mature females from the different experimental groups are very stable; a higher variability among treatments is noted for the measured values of hepatopancreatic lipid ($\pm 30\%$ in control versus $\pm 40\%$ in the *Artemia* treatments). For both tissues the values in mature females are markedly superior to the ones measured before the onset of

Table 4
Experiment 2: Lipid content (% of dry weight) and levels of selected FA (area% of total FA) in broodstock dietary ingredients, in hepatopancreas and ovaries of *P. vannamei* matured under different dietary conditions, and in nauplius five stages collected from captive broodstock and wild spawners

	Lipid	EPA	DHA	Σ PUFA	Σ(n−3)	Σ(n−6)	DHA/ EPA	Σ(n−3)/ Σ(n−6)
Broodstock dietary ingredients								
Food mixture	12.1	10.41	12.37	45.63	37.34	8.29	1.19	4.50
Panama bloodworm	11.5	8.60	4.20	44.45	29.88	14.57	0.49	2.05
Artemia	12.7	7.63	1.45	25.90	15.72	10.18	0.19	1.54
Enriched *Artemia*	17.1	7.89	1.61	23.72	13.52	10.20	0.20	1.33
Immature animals								
Hepatopancreas	24.5	9.08	17.27	47.47	37.16	10.31	1.90	3.60
Ovary	9.0	10.66	8.72	42.68	29.18	15.31	0.82	1.79
Mature animals								
Hepatopancreas								
Control	29.6	8.14	8.67	40.89	31.79	9.10	1.07	3.49
Art	40.1	6.61	6.68	36.17	27.46	8.71	1.01	3.15
ArtE	39.4	8.75	8.60	38.80	30.67	8.13	0.98	3.77
Ovary								
Control	22.8	8.57	11.38	34.89	27.94	6.95	1.33	4.02
Art	20.0	9.47	9.22	33.27	25.63	7.64	0.97	3.35
ArtE	19.1	9.10	9.74	32.45	24.88	7.57	1.07	3.29
Nauplius 5								
Maturation in the natural environment	13.6	9.73	7.17	38.09	25.84	12.45	0.74	2.06
Maturation in captivity								
Control	20.1	9.43	10.07	35.96	27.57	8.40	1.07	3.28
Art	19.2	7.59	7.14	32.32	21.26	11.05	0.94	1.92
ArtE	16.0	7.67	6.43	30.71	21.21	9.50	0.83	2.23

For explanation of the abbreviations it is referred to the text.

Table 5
Experiment 3: Increase of reproductive performance of female *P. vannamei* broodstock under commercial conditions when a standard broodstock diet mixture of fresh frozen marine organisms is partially substituted with enriched *Artemia* biomass

Reproduction characteristic	Increase (%)
All females spawning (%)	83
Spawn size	18
Hatching percentage	10
Nauplii per spawn	30
Nauplii per female	138

the maturation process ($\pm 25\%$). The proportions of polyunsaturated fatty acids (PUFA) are highest in the food mixture and the bloodworm (45.6% resp. 44.5%); the *Artemia* preparates are substantially lower (26.9% and 23.7% in non-enriched, respectively enriched *Artemia*). Striking differences in DHA ($22{:}6n-3$) were found with measured values of 12.4% in the mixture, 4.2% in the bloodworm and around 1.5% in *Artemia*. For the EPA component ($20{:}5n-3$), the variation is much less with values ranging between 7.6 and 10.4%. As a consequence, DHA/EPA ratios reach only values of about 0.5 in the control diet and 0.2 in the *Artemia* diets. A selective migration of DHA towards the ovaries is observed when the females mature, and seems to be correlated with the amount of DHA ingested as it is most relevant in the control treatment. It is furthermore observed that due to these high concentrations of DHA the DHA/EPA ratio is superior to the one observed in nauplii from wild spawners.

3.3. Experiment 3

A summary of the results is presented in Table 5. The reproductive data obtained in the *Artemia* fed tanks are markedly higher than for the control tanks, especially for the number of females that reproduce daily and the number of nauplii obtained per spawn. Expressed as a percentage of those recorded in the tanks offered the control diet the *Artemia* fed tanks showed an 83% increase of the mature females that could be inseminated. Also spawn size and hatching percentage increased by 18% and 10%, respectively. Consequently the number of nauplii per spawn in the treatments receiving the enriched *Artemia* supplementation increased almost 30%.

4. Discussion

Possible effects due to individual variation among experimental animals were reduced by randomly stocking the tanks. Environmental and system factors other than the diet that can affect the reproduction of penaeid shrimp are numerous and are reviewed by Bray and Lawrence (1992). However, within each of the present experiments such factors can safely be considered either constant for all tanks (e.g. temperature and quality of the incoming water, photoperiod and light intensity, tank size, tank shape) or acting randomly on the different tanks (e.g. noise and disturbances due to operational procedures) and therefore are unlikely to have contributed to the observed differences.

Survivals in the experimental trials differed markedly and may be explained in part by the history of the broodstock. In the CENAIM-trial, the experimental animals were collected from the natural environment just prior to the start of the test and a rather severe selection occurred during the acclimation period immediately preceding the experiment singling out the fittest animals. In contrast, the poor survival at WMC may have resulted from a combination of several factors including the technical error described in the results section. At the start of the experiment the broodstock were over 12 months old as they went through an extended overwintering and holding phase. Although every effort was made to minimize stress during tank transfers, some handling mortality was observed. These factors may have been the most significant as evidenced by the equally poor survival of the males. An additional stress on the females resulted from the twice daily sourcing carried out four times per week. All of these factors may well have contributed to reduce survival and produce the relatively slow reproductive performance observed across all three treatments.

The production capacity of a maturation facility depends on the combined performance of both sexes: potency and ability of the males to transfer spermatophores, maturation rate of the females, degree of fertilization, and egg/larval quality. From the WMC-experiment it appears that the effect of the male diet on mating and fertilization success is insignificant: males fed squid performed as well or better than males fed *Artemia* or bloodworm supplements. The mixed sex trials at CENAIM did not allow specific evaluation of the male contribution to reproductive success. However it may be assumed that the differences in the maturation output were not specifically related to male performance since known factors such as inadequate sex ratio and male impotence were absent in all treatments. At stocking, the male-to-female ratio in all treatments was close to 1:1 and remained within the optimal range of 1–1.5:1 (Bray and Lawrence, 1992) during the course of the experiment. Furthermore it is assumed that only males with fully developed spermatophores participate in the reproduction process. Leung-Trujillo and Lawrence (1991) report a sperm regeneration time for *P.vannamei* of 2 to 4 days and cite nutrition among the possible factors interfering, but it is thought unlikely that the dietary impact on sperm regeneration is so drastic that under conditions of average daily male to mature female ratios of around 4, shortage of ripe males was the basis for poor mating success. Another argument to exclude male inefficiency as a possible explanation for the varying production figures between treatments is that unfertilized eggs were rarely detected in the outflow of the maturation tanks.

On the other hand all three tests clearly demonstrate that female reproductive performance is affected by the broodstock diet. In particular, maturation, mating and fertilization success is improved when bloodworm or *Artemia* biomass forms part of the diet, resulting in a higher number of females spawning and a higher number of nauplii produced per unit. Effects on spawn quality were more difficult to quantify due to the high variances and further study is necessary to evaluate possible effects on naupliar fitness.

Nutrition has been repeatedly emphasized as a key factor for successful maturation of captive broodstock (reviews of Harrison, 1990; Bray and Lawrence, 1992; Browdy, 1992; and Primavera, 1985). Recommended feeding regimes typically consist of a combination of fresh or fresh-frozen and dry formulated rations. Commonly used natural

food organisms include squid, mussels, clams, oysters, crustaceans and polychaetes. The relatively poor performance of the squid fed tanks in the WMC-experiment confirms earlier studies suggesting that diets made up of combinations of fresh-frozen marine food organisms have outperformed diets composed of any of the components fed singly. Chamberlain and Lawrence (1981) report that a squid diet outperformed shrimp, clam or worms fed separately. However, the combination of feeds yielded the best reproductive performance, outperforming any of the diets fed separately. Galgani et al. (1989) report that for *P.vannamei* a diet composed of molluscs and fish gave best results along with natural food supplemented with a prepared diet. Bray et al. (1990) report that diets with multiple fresh components including squid, bloodworms, shrimp and brine shrimp performed better than sole squid diets. In the WMC-experiment, the reduced reproductive performance of females in the control tanks as compared to mixed diets in other WMC-treatments and in CENAIM-tanks, further illustrates the importance of feed quality.

This is further confirmed by comparing the bloodworm and *Artemia* treatments in the WMC- (treatment 2 and 3) and CENAIM-tests (treatment 1 and 2). The latter experiment shows more significant differences between control diets and *Artemia* supplemented tanks. This may be explained by the type of bloodworm that was administered: i.e. for the CENAIM-test *A. reseii* from Panama was used, which is generally accepted among hatchery managers to be of a lower quality compared to the Maine polychaetes (*G. dibranchiata*) used in the WMC-trials.

It remains unclear, however, which is the specific dietary component of the marine polychaete or brine shrimp that enhances the maturation performance in penaeid shrimp. Many studies have focused on the lipid fraction as an energy source and providing the necessary nutrients for continuous maturation and synthesis of reproductive tissue (Middleditch et al., 1979, 1980; Teshima and Kanazawa, 1983). Fully developed ovaries contain large amounts of lipids, i.e. up to 10% of the female's wet body weight (Bray et al., 1990). The capacity of the digestive gland to act as a lipid store is considered limited, suggesting a strong dependence on dietary lipid (Castille and Lawrence, 1989). By varying the lipid content of a dry formulated diet that was fed in combination with a fresh-frozen component to *P. stylirostris*, Bray et al. (1990) found that total lipid levels of 7.8% and 11.1% produced better results than 13.9%. Moreover, shrimp possess only limited or no ability to synthesize long chain highly unsaturated fatty acids (HUFA) or cholesterol, although both components are major constituents in crustacean eggs and gonadal tissues (Harrison, 1990).

Millamena et al. (1986) reported an improved reproductive performance when the broodstock diet of *P. monodon* was supplemented with cod liver oil as a source of $n-3$ HUFA. However, the present study in CENAIM does not confirm the latter observation since *Artemia* supplementation reduced the overall $n-3$ HUFA content of the broodstock diet but increased performance (Table 5). It is therefore unlikely that $n-3$ HUFA, or more specifically DHA, are the nutrients that further enhance the maturation performance in our tests.

In comparing *Artemia* treatments in the CENAIM-experiment, brine shrimp biomass enriched with $n-3$ HUFA did not perform better than the non-boosted diet. However, one should be very careful with the interpretation of these data since the biochemical

analysis was only of one batch of enriched *Artemia* and did not reveal any differences in fatty acid composition compared to the non-enriched brine shrimp biomass. Moreover, other factors may have interfered here, such as suboptimal processing and storage under local conditions, inadequate dosage of the experimental booster, or even inadequate formulation of it (e.g. very high levels of vitamin E (3000 ppm) were incorporated, which may have created effects of oversaturation). More research is needed to evaluate effects of enriched versus non-enriched *Artemia* biomass and to identify potentially active ingredients and their optimal doses.

Another possible factor in *Artemia* adults that may trigger reproduction in penaeid shrimp could be of endocrine origin. It is well known that a number of specific hormones (stimulatory as well as inhibitory) play a role in the reproduction process in all shrimp species (Mzusy and Payen, 1988). Recent research has furthermore suggested that oral administration of specific neuropeptides may stimulate maturation in insects and possibly, crustaceans (Schoofs, pers. comm.). It is not known if reproductively active adult brine shrimp may contain analogous peptides that could be active in promoting reproduction in heterologous species.

5. Conclusions

The results of the present study show that *Artemia* biomass is a potential candidate for supplementation or replacement of bloodworm in maturation diets for *P.vannamei*. Even in a maturation diet consisting of multiple natural food products, frozen adult brine shrimp appears to be a performing ingredient. The results also suggest that the dietary effect of *Artemia* may be attributed more to female performance than to male performance. In view of the overall good performance of *Artemia* biomass when supplemented in broodstock diet, its lower cost and the more stable supply (Lavens and Sorgeloos, 1996), frozen adult brine shrimp appears to be a valid commercial alternative for marine polychaetes in *P. vannamei* maturation diets. Moreover, *Artemia* adults can be used as a carrier for various nutrients or other components, which may further enhance reproductive characteristics and quality of offspring.

6. Unlinked reference

Albreksten et al., 1988

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